Peritubular capillaritis was more frequently seen in severe GVHD. These results indicated the association between peritubular capillaritis and GVHD, and are consistent with the previous animal studies [28]. However, it remains unclear whether peritubular capillaritis results from GVHD, or from medications and infection, and further investigation is required. Lower rates of glomerulitis in bone marrow recipients compared to renal transplants may be due to differences of patients' characteristics between these two groups. Subjects who received renal transplants may tend to develop glomerulitis after transplants because of their primary diseases including glomerulonephritis [29, 30].

Our study suggests that kidney can be a target of GVHD and that Banff criterion is a useful tool to access renal pathology after allo-SCT. We believed that our finding will be helpful to clinicians, but there are several limitations to be discussed. First, the study was retrospective and small in size. Second, autopsy findings may not show active changes due to suppressed immune system postmortem. However, routine follow-up renal biopsy after renal transplants is not realistically plausible. In order to evaluate microscopic changes, we could only use autopsy pathology. Future study would understand this limitation and examine more autopsy cases to assess the association between GVHD and renal damages.

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blood

2008 112: 2579-2582
Prepublished online Jun 16, 2008;
doi:10.1182/blood-2007-11-118893

Chronic graft-versus-host disease following umbilical cord blood transplantation: retrospective survey involving 1072 patients in Japan

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published semimonthly by the American Society of Hematology, 1900 M St, NW, Suite 200, Washington DC 20036.

Brief report

Chronic graft-versus-host disease following umbilical cord blood transplantation: retrospective survey involving 1072 patients in Japan

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We have little information on chronic graft-versus-host disease (GVHD) after cord blood transplantation (CBT). We investigated its clinical features in 1072 Japanese patients with hematologic malignancies who received a transplant through the Japan Cord Blood Bank Network. The primary end point was to investigate the incidence of any chronic GVHD. Median age of the patients was 33 years (range, 0-79 years). The cumulative incidence of chronic GVHD 2 years after transplantation was 28%. Chronic GVHD was fatal in 29 patients. Multivariate analysis demonstrated that development of chronic GVHD was favorably associated with both overall survival and event-free survival. Multivariate analysis identified risk factors of chronic GVHD: higher patient body weight, higher number of mismatched antigens for GVHD direction, myeloablative preparative regimen, use of mycophenolate mofetil in GVHD prophylaxis, and development of grades II to IV acute GVHD. Although chronic GVHD is a significant problem after CBT, it is associated with improved survival, perhaps due to graft-versus-malignancy effects. (Blood. 2008;112:2579-2582)

Introduction

Chronic graft-versus-host disease (GVHD) is a significant concern in allogeneic hematopoietic stem cell transplantation (HSCT). Many studies have been published on clinical features of chronic GVHD following bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT). In contrast, we have limited information on chronic GVHD following cord blood transplantation (CBT).

Methods

Informed consent was obtained in accordance with the Declaration of Helsinki. According to local policy, the study was approved by Japan Cord Blood Bank Network. We had no direct contact with human subjects during our study; data on patients who underwent CBT were obtained from the Japan Cord Blood Bank Network. The primary end point of this study was to investigate the incidence of any chronic GVHD after CBT. Cord blood units are provided with written informed consent. Between June 1997 and August 2006, 2713 cord blood transplant recipients were registered with the Japan Cord Blood Bank Network. All recipients received a single cord blood unit. We included those with hematologic malignancies who underwent CBT without T-cell depletion. We excluded patients with a history of any type of allogeneic HSCT prior to CBT. A total of 2015 patients met the criteria. Of those, we excluded 943 with disease progression, death without progression, and graft failure within 100 days after transplantation. In this study, we retrospectively investigated clinical features of chronic GVHD in the remaining 1072 patients. Cytomegalovirus-seropositive cord blood unit was not provided to transplantation centers. Acute and chronic GVHD were diagnosed and graded according to standard criteria.[1,2] GVHD that developed after day 100 was defined as chronic GVHD. If the mode of presentation for chronic GVHD was progressive, the date of onset was defined as day 100. SAS version 9.1.3 (SAS Institute, Cary, NC) was used for all statistical analyses.

Results and discussion

The median age of the 1072 patients was 33 years (range, 0-79 years) and the median patient body weight was 50.0 kg (range, 4.0-96.1 kg). The median follow up of the surviving patients was 18.1 months (range, 3.3-110.1 months). Of the 1072 patients, 492 (46%) developed grades II to IV acute GVHD within 100 days of transplantation. Chronic GVHD was diagnosed in 312, and the cumulative incidence of chronic GVHD 2 years after transplantation was 28% (95% confidence interval [CI], 25%-31%; Figure 1).

Submitted November 2, 2007; accepted January 19, 2008. Prepublished online as *Blood* First Edition paper, June 16, 2008; DOI 10.1182/blood-2007-11-118893.

The online version of this article contains a data supplement.

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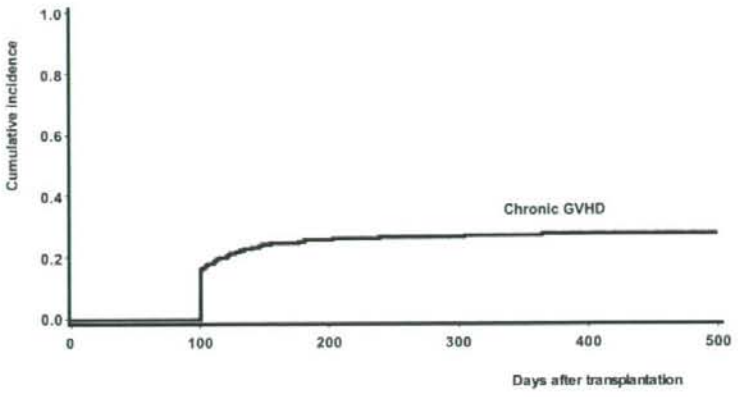

Figure 1. Cumulative incidence of chronic GVHD. The cumulative incidence of chronic GVHD 2 years after transplantation was 28% (95% confidence interval [CI], 25%-31%). Cumulative incidence curves were used to calculate the probability of chronic GVHD to take account of competing risks: disease progression, death without disease progression, and onset of graft failure.

Of the 299 patients in whom the information on types of chronic GVHD was available, 184 and 115 patients developed limited and extensive diseases, respectively.

The onset of chronic GVHD was quiescent (n = 225), de novo (n = 48), progressive (n = 18), and unknown (n = 8). Information on involved organs of chronic GVHD was available in 145 patients. Their involved organs were skin (n = 94), oral cavity (n = 35), liver (n = 32), lung (n = 24), gastrointestinal tract (n = 20), and eye (n = 16). Of 224 patients from whom information on both the treatment of chronic GVHD and its response was available, 93 patients (42%) received systemic immunosuppressive agents including corticosteroids (n = 63), calcineurin inhibitors (n = 15), both (n = 13), and others (n = 2). Of those 93 patients, 64 patients (68%) responded to the treatment. Chronic GVHD was refractory in the remaining 29, and fatal in 5.

Multivariate analysis identified prognostic factors of chronic GVHD: higher patient body weight (hazard ratio [HR], 1.01; 95% CI, 1.01-1.02; $P = .001$), higher number of mismatched antigens for graft-versus-host direction (HR, 1.31; 95% CI, 1.07-1.60; $P = .01$), reduced-intensity preparative regimen (HR, 0.69; 95% CI, 1.11-3.28, $P = .02$), use of mycophenolate mofetil in GVHD prophylaxis (HR, 1.91; 95% CI, 1.11-3.28, $P = .02$), and development of grades II to IV acute GVHD (HR, 1.51; 95% CI, 1.18-1.93; $P = .001$; Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Of the 1072 patients, 307 died during follow up due to disease progression (n = 189), chronic GVHD (n = 29), infection (n = 23), and others (n = 66). Prognostic factors of transplantation outcomes are shown in Table 1. Overall survival (OS) and event-free survival (EFS) at 3 years after CBT were 63% (95% CI, 36%-90%) and 54% (95% CI, 27%-80%), respectively.

The present study is the first larger report addressing features of chronic GVHD in CBT. The incidence of chronic GVHD after CBT was 28% in our study, which was comparable with the previous smaller reports on CBT conducted in Western countries[3-6] and was lower than that after BMT from unrelated donors (UR-BMT) in Japanese patients.[7,8] Since the genetic backgrounds of the Japanese people are relatively homogeneous compared with those of the Western people, the incidences of chronic GVHD following UR-BMT are reportedly low in Japanese patients; our results in CBT contrast with such previous investigations.[7,8] The present study suggests that the mechanism of GVHD development is possibly affected by factors other than the genetic backgrounds of the patients' ethnicities. There are few previous studies on treatment responses of chronic GVHD after CBT. The present study suggests a higher response rate and lower mortality in chronic GVHD patients after CBT than that after UR-BMT and PBSCT.[9-16] The low overall incidence despite HLA mismatches, relatively low need for systemic immunosuppression, and positive survival impact indicate milder nature of chronic GVHD after CBT. This may be related to the immature lymphocytes in cord blood that are immunologically tolerant,[17,18] although the basic mechanism is unknown and awaits further investigations.

The present study demonstrated that chronic GVHD after CBT improved EFS and reduced the risk of disease progression. Although graft-versus-malignancy (GVM) effects of chronic GVHD after CBT have not been reported, our results were comparable with previous studies in BMT and PBSCT.[2,19,20] Chronic GVHD following CBT probably has clinically significant GVM effects. Interestingly, chronic GVHD after CBT improved OS as well. These findings contrasted with the unclear effects of chronic GVHD on OS improvement in conventional allogeneic HSCT.[2,19,20] The observations are probably associated with the possible favorable impact of chronic GVHD after CBT on nonrelapse mortality.

The present study provided useful information on chronic GVHD after CBT, although some issues remain to be discussed. First, since our study was retrospective, unrecognized bias may have affected the results. Second, we used the classical diagnostic criteria of chronic GVHD[2] and did not distinguish the late onset acute GVHD that occurs after day 100 from the classic chronic GVHD as the National Institutes of Health consensus project proposed.[21] Third, suggestions by our study on future strategies for cord unit selection and GVHD prophylaxis would be informative. Our multivariate analysis suggested an association of mycophenolate mofetil with development of chronic GVHD, whereas methotrexate was associated with better nonrelapse mortality. Although the mechanism is uncertain, clinicians may prefer to use methotrexate as GVHD prophylaxis. Since GVM effects of chronic GVHD were suggested, risk stratification may be possible in cord blood selection. Using cord blood with higher HLA mismatches for patients with high-risk primary diseases might be permissible. Finally, the applicability of our results to patients with higher body weight with heterogeneous genetic backgrounds in Western countries remains to be discussed. All of our patients received single cord blood units, whereas the majority of cord blood transplants are double cord blood units in Western countries. However, the incidence of chronic GVHD is lower and response to immunosuppressants is

Table 1. Risk factors of overall survival and event-free survival in multivariate analysis

	Overall survival			Event-free survival†			Disease progression			Nonrelapse mortality‡		
Multivariate factors*	HR 95%	CI	P	HR 95%	CI	P	HR 95%	CI	P	HR 95%	CI	P
Patient age, 40 y or older vs younger than 40 y	1.80	1.32-2.44	.0002	1.72	1.36-2.17	<.001	‡‡			2.76	1.86-4.10	<.001
Primary diseases§												
Acute myeloid leukemia	1.00			1.00			1.00			‡‡		
Acute lymphoblastic leukemia	1.42	1.03-1.97	.03	1.30	0.99-1.70	.06	1.14	0.84-1.54	.40	‡‡		
Adult T-cell leukemia/lymphoma	1.38	0.72-2.64	.33	1.54	0.87-2.72	.14	1.83	0.91-3.67	.09	‡‡		
Myelodysplastic syndrome	0.76	0.52-1.11	.16	0.65	0.46-0.91	.01	0.58	0.39-0.86	.01	‡‡		
Chronic myelogenous leukemia	0.50	0.24-1.04	.07	0.64	0.38-1.11	.11	0.61	0.32-1.14	.12	‡‡		
Non-Hodgkin lymphoma	0.82	0.53-1.26	.36	0.86	0.61-1.23	.42	0.84	0.55-1.29	.42	‡‡		
Hodgkin lymphoma	2.44	0.73-8.16	.15	3.41	1.35-8.58	.01	3.58	1.11-11.51	.03	‡‡		
Multiple myeloma	0.59	0.28-1.25	.17	0.79	0.42-1.49	.46	0.77	0.34-1.78	.54	‡‡		
Disease status at transplantation‖												
Early¶	1.00			1.00			1.00			‡‡		
Intermediate#	1.15	0.78-1.71	.49	1.09	0.79-1.52	.60	1.11	0.76-1.62	.60	‡‡		
Advanced**	2.44	1.78-3.35	<.001	2.32	1.78-3.02	<.001	2.65	1.94-3.62	<.001	‡‡		
ABO mismatch												
Match	1.00						‡‡			‡‡		
Major mismatch	0.69	0.50-0.94	.02	‡‡			‡‡			‡‡		
Minor mismatch	0.88	0.63-1.22	.44	‡‡			‡‡			‡‡		
Major/minor mismatch	1.10	0.79-1.54	.56	‡‡			‡‡			‡‡		
Higher no. of serologic HLA disparity for host-versus-graft direction, linear by 1 antigen increase	‡‡			0.80	0.69-0.92	.002	0.79	0.67-0.92	.004	‡‡		
Sex of the donor, female vs male	‡‡			‡‡			1.35	1.06-1.72	.02	‡‡		
Preparative regimen, reduced-intensity vs myeloablative regimen	1.25	0.92-1.70	.15	‡‡			‡‡			‡‡		
Methotrexate use in GVHD prophylaxis	‡‡			‡‡			‡‡			0.58	0.39-0.86	.01
Acute GVHD††												
Grades 0-I	1.00			1.00			‡‡			1.00		
Grade II	0.95	0.72-1.26	.73	0.85	0.67-1.08	.18	‡‡			1.26	0.79-2.02	.33
Grades III-IV	1.77	1.29-2.43	.001	1.37	1.03-1.81	.03	‡‡			3.66	2.31-5.81	<.001
Chronic GVHD	0.71	0.54-0.94	.02	0.64	0.50-0.82	.001	0.65	0.48-0.87	.003	0.66	0.42-1.03	.07

Associations between potential prognostic factors and outcomes were evaluated using Cox proportional hazard regression models. Occurrence of chronic GVHD was included into models as a time-dependent covariate. We used a stepwise procedure at a significance level of 5% to construct prognostic models. The proportional hazards assumption of Cox model was assessed mainly by a graphic approach.

HR indicates hazard ratio; 95% CI, 95% confidence interval; and GVHD, graft-versus-host disease.

*The following variables were considered in univariate analysis: recipient age at transplantation; recipient body weight; HLA mismatch; blood type mismatch; sex mismatch; infused nuclear cell dose and $CD34^+$ cell dose; primary diseases; stage of primary diseases at transplantation; previous history of hematopoietic transplantation; preparative regimen and GVHD prophylaxis; and previous acute GVHD.

†EFS was defined as the time from transplantation to the following events: first progression of the primary diseases, death without progression, and graft failure. Progressions of leukemia and myelodysplastic syndrome were defined as relapse of primary diseases on the basis of morphologic evaluation in the bone marrow or other sites. Progression of malignant lymphoma was defined as involvement of new sites, recurrence in originally involved sites, or increase of more than 25% in original tumor masses. Progression of multiple myeloma was defined as an increase in serum or urine M-component, development of new soft tissue plasmacytomas, and/or increase in the size of soft tissue plasmacytomas.

‡ Nonrelapse mortality was defined as death without progression of the primary diseases.

§Those included acute lymphoblastic leukemia (n = 347), acute myeloid leukemia (n = 323), adult T-cell leukemia (n = 28), myelodysplastic syndrome (n = 171), chronic myelogenous leukemia (n = 50), non-Hodgkin lymphoma (n = 117), Hodgkin lymphoma (n = 7), and multiple myeloma (n = 29).

‖Disease status at CBT was classified into 3 groups according to the standardized report forms of the International Bone Marrow Transplant Registry (IBMTR).

¶The early group including first complete remission of acute leukemia and lymphoma, first chronic phase of chronic myeloid leukemia, first complete or partial remission of multiple myeloma, and myelodysplastic syndrome refractory anemia.

#The intermediate group including second or subsequent complete remission of acute leukemia and lymphoma, accelerated phase of chronic myeloid leukemia, and second or subsequent complete or partial remission of multiple myeloma.

**The advanced group including refractory disease, relapse or partial response of acute leukemia and lymphoma, blastic crisis of chronic myeloid leukemia, and other malignancies.

††Those included grade 0 (n = 252), grade I (n = 297), grade II (n = 328), grade III (n = 148), and grade IV (n = 16).

‡‡Those factors were not included in the multivariate prognostic models because those *P* values did not exceed .05 in univariate analysis.

better in CBT than in UR-BMT in Western countries[22]; hence, the milder nature with lower incidence in chronic GVHD following CBT is probably common among Japanese and Westerns patients. The features of chronic GVHD following CBT in Western adult patients require further investigations.

In conclusion, the present study demonstrated that the incidence of chronic GVHD after CBT is probably lower than BMT or PBSCT, and is associated with improved survival. More intensive studies on the biology of chronic GVHD in CBT are warranted for our understanding and better management of chronic GVHD.

Acknowledgments

We are grateful to Dr Takanori Teshima at the Center for Cellular and Molecular Medicine of Kyushu University Hospital (Fukuoka, Japan) for providing useful information.

This work was supported by a Research Grant on Tissue Engineering (H17-14) from the Ministry of Health, Labor and Welfare of Japan.

Authorship

Contribution: H.N., S.M., and M.K. designed the research and wrote the paper; T.Y. analyzed data; T.M., K.Y., N.M., E.K., Y. Kodama, T.K., H.S., Y. Kouzai, M.O., Y.O., R.K., M.I., and S. Takahashi performed research; S. Kai, K.K., T.I.-N., and S. Taniguchi managed the data; and S. Kato took leadership of the authors.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Journal of Antimicrobial Chemotherapy (2008) **61**, 411–416
doi:10.1093/jac/dkm463
Advance Access publication 30 November 2007

JAC

False-positive *Aspergillus* galactomannan antigenaemia after haematopoietic stem cell transplantation

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Received 2 September 2007; returned 8 October 2007; revised 2 November 2007; accepted 4 November 2007

***Objectives*: Although *Aspergillus* galactomannan (GM) antigen detection is widely applied in the diagnosis of invasive aspergillosis (IA), false-positive reactions with fungus-derived antibiotics, other fungal genera or the passage of dietary GM through injured mucosa are a matter of concern. The aim of this study was to investigate the cumulative incidence and risk factors for false-positive GM antigenaemia.**

***Patients and methods*: The records of 157 adult allogeneic haematopoietic stem cell transplantation (HSCT) recipients were retrospectively analysed. Episodes of positive GM antigenaemia, defined as two consecutive GM results with an optical density index above 0.6, were classified into true, false and inconclusive GM antigenaemia by reviewing the clinical course.**

***Results*: Twenty-five patients developed proven or probable IA with a 1 year cumulative incidence of 12.9%, whereas 50 experienced positive GM antigenaemia with an incidence of 32.2%. Among the total 58 positive episodes of the 50 patients, 29 were considered false-positive. The positive predictive value (PPV) was lower during the first 100 days than beyond 100 days after HSCT (37.5% versus 58.8%). Gastrointestinal chronic graft-versus-host disease (GVHD) was identified as the only independent significant factor for the increased incidence of false-positive GM antigenaemia (PPV 0% versus 66.7%, $P = 0.02$).**

***Conclusions*: GM antigen results must be considered cautiously in conjunction with other diagnostic procedures including computed tomography scans, especially during the first 100 days after HSCT and in patients with gastrointestinal chronic GVHD.**

Keywords: fungal infections, invasive aspergillosis, chronic GVHD, gastrointestinal tract, mucosal damage

Introduction

Invasive aspergillosis (IA) remains one of the leading infectious causes of death after allogeneic haematopoietic stem cell transplantation (HSCT), despite new antifungal agents that have become available in recent years.[1] The high mortality rate of IA was mainly attributed to the difficulty of diagnosis at the early stage of the disease, because histopathological examinations require invasive procedures and fungal cultures have low specificity and sensitivity in detecting IA.

Monitoring of the circulating *Aspergillus* galactomannan (GM) antigen by the sandwich enzyme-linked immunosorbent assay (ELISA) is a feasible non-invasive biological method for early diagnosis of IA.[2] The GM ELISA test has sensitivity of 67% to 100% and specificity of 81% to 99% in neutropenic patients and allogeneic transplant recipients,[3–6] and was introduced as microbiological evidence in the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria for opportunistic invasive fungal infection.[7] However, a concern is the false-positive reactions,

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which may lead to inappropriate invasive investigation or over-treatment with antifungal agents. Previous studies have reported various risk factors for the false-positive results, including early childhood,[3] the development of chronic graft-versus-host disease (GVHD),[8] the passage of GM of food origin[9,10] and certain exoantigens from other fungal genera[11] or fungus-derived antibiotics.[12,13] However, little is known about the exact mechanism of false-positive reactions with these factors.

To clarify the cause of false-positive results, we retrospectively analysed the incidence and risk factors for false-positive GM antigenaemia in allogeneic HSCT recipients.

Patients and methods

Study population

GM ELISA became available at the University of Tokyo Hospital as a routine diagnostic test in February 2000. During a 5 year period (February 2000 to May 2005), 163 consecutive adult patients (>16 years old) underwent allogeneic HSCT at the University of Tokyo Hospital. The medical records of 157 patients who had at least two GM ELISA tests after HSCT were available for a retrospective analysis of positive GM antigenaemia. The median follow-up was 519 days (range, 15–2090 days) after HSCT. The patient characteristics are shown in Table 1. Acute leukaemia in first remission, chronic myelogenous leukaemia in first chronic phase, myelodysplastic syndrome with refractory anaemia or refractory anaemia with ringed sideroblasts, and aplastic anaemia were defined as low-risk diseases, whereas others were considered high-risk diseases. Donors other than human leucocyte antigen (HLA)-matched sibling donors were defined as alternative donors.

Transplantation procedure

The conventional preparative regimen for leukaemia/lymphoma was mainly performed with either cyclophosphamide/total body irradiation (TBI)-based regimens or busulfan/cyclophosphamide-based regimens. In cyclophosphamide/TBI-based regimens, the dose of cyclophosphamide was decreased and etoposide was added instead in patients with impaired cardiac function. Fludarabine-based regimens were used as reduced-intensity regimens for elderly or clinically infirm patients.[14] Cyclosporin A or tacrolimus was administered combined with short-term methotrexate for prophylaxis against GVHD. Alemtuzumab was added for patients who received a graft from an HLA-mismatched donor.[15] Methyl-prednisolone or prednisolone at 1 or 2 mg/kg was added for patients who developed grade II–IV acute GVHD, whereas prednisolone at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, herpes simplex virus and *Pneumocystis jirovecii* infections consisted of tosufloxacin, aciclovir and sulfamethoxazole/trimethoprim.

Antigen detection

GM assay was performed at least every other week after HSCT until discharge from the hospital in the majority of patients. In the outpatient setting, the monitoring of GM was continued at each visit in patients who were receiving immunosuppressive therapy, at the discretion of attending physicians. Circulating *Aspergillus* GM was detected using a sandwich immunocapture ELISA (Platelia Aspergillus, Bio-Rad, Marnes-la-Coquette, France) using a rat anti-GM monoclonal antibody.[2] The technique was performed as recommended by the manufacturer. The optical absorbance of specimens and controls was determined with a spectrophotometer set at 450 and 620 nm wavelengths. The optical density (OD) index for each sample was calculated by dividing the optical absorbance of the clinical sample by that of the threshold control. Two consecutive serum samples with an OD index of 0.6 or more were considered positive.[16]

Table 1. Patients' characteristics

Characteristic	Total patients
Sex (male/female)	105/52
Age, median (range)	41 (16–66)
Underlying disease	
acute leukaemia	70
CML	26
MDS	22
SAA	8
other	31
Graft source	
PBSC	69
BM	88
Donor type	
matched sibling	58
mismatched related	15
unrelated	84
Preparative regimen	
Cy (Etp)/TBI-based regimens	105
Bu/Cy-based regimens	15
ATG-based regimens for SAA	5
Flu-based RIC	32
GVHD prophylaxis	
CsA+MTX	115
tacrolimus+MTX	18
alemtuzumab+CsA+MTX	24
Acute GVHD	
grade 0–I	87
grade II–IV	69
Chronic GVHD	
extensive	57
limited	30
none	47

CML, chronic myelogenous leukaemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anaemia; PBSC, peripheral blood stem cell; BM, bone marrow; Cy, cyclophosphamide; Etp, etoposide; TBI, total body irradiation; Bu, busulfan; ATG, antithymocyte globulin; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporin A; MTX, methotrexate.

Antifungal prophylaxis and treatment for IA

As antifungal prophylaxis, fluconazole at 200 mg was principally given daily from day −14 until the end of immunosuppressive therapy. For patients with a history of IA, intravenous micafungin at 150–300 mg or oral itraconazole at 200 mg was administered instead. All patients were isolated in high-efficiency particulate air (HEPA)-filtered rooms from the start of the conditioning regimen to engraftment. Febrile neutropenia was treated with broad-spectrum antibiotics in accordance with

the published guidelines.[17] Antifungal treatment was started when febrile neutropenia persisted for at least 3–4 days or when IA was confirmed or suspected with clinical or radiological signs.

Diagnosis procedures and definitions

Diagnostic procedures included routine cultures of urine and stools, repeated cultures of blood and sputum, weekly chest X-ray, computed tomography (CT) scan of the chest and nasal sinus and, when possible, bronchoscopic examinations and open biopsy. CT scans were principally obtained for patients with (i) clinical signs and/or symptoms suggestive of IA, (ii) persistent or recurrent febrile neutropenia while on broad-spectrum antibiotic treatment, (iii) infiltrates or nodules on chest X-ray or (iv) positive GM antigenaemia. In patients with clinical suspicion of IA, bronchoscopy with bronchoalveolar lavage (BAL) and/or tissue biopsy were also performed whenever feasible. A diagnosis of IA was classified as proven or probable on the basis of the EORTC/MSG definitions.[7] True-positive GM antigenaemia was defined as two consecutive positive results with the established diagnosis of proven or probable IA. Positive GM antigenaemia in episodes that did not fulfil the diagnostic criteria for proven or probable IA was considered as inconclusive-positive if (i) sufficient examinations including chest and/or sinus CT scans were not performed despite the presence of compatible clinical signs and symptoms of IA or (ii) the possibility that the radiological abnormalities on the CT scans were due to IA could not be denied because of the use of empirical antifungal therapy or targeted antifungal therapy for other definite fungal infections at the time of positive antigenaemia. Alternatively, positive antigenaemia without sufficient evidence to diagnose proven or probable IA was considered as false-positive in any of the following: (i) no radiological abnormalities were detected on chest and/or sinus CT scans; (ii) non-specific abnormalities on CT scans improved without any antifungal treatments for IA or culture results for specimens from radiologically abnormal sites including BAL fluid or sinus aspirate were negative; or (iii) CT scans were not performed because of no evidence meeting clinical minor criteria in EORTC/MSG definitions. Positive antigenaemia recurring after the negative conversion at least 3 months apart was considered an independent episode.

Statistical analysis

Sensitivity, specificity and positive predictive value (PPV) of the GM ELISA were calculated on the basis of the clinical diagnosis of proven or probable IA. The cumulative incidences of positive GM antigenaemia and IA were evaluated using Gray's method, considering death without each event as a competing risk.[18] Probabilities in two groups were compared using Fisher's exact test. P values of less than 0.05 were considered statistically significant.

Results

Transplantation outcome

One hundred and fifty-seven allogeneic transplant recipients were included in the study. Neutrophil engraftment was obtained at a median of 17 days (9–43 days) after HSCT in 156 patients. Grade II–IV acute GVHD was observed in 69 and chronic GVHD in 87 of 134 who survived more than 100 days. Seventy patients died, the causes being haematological relapse ($n = 29$), infection ($n = 14$), non-infectious pulmonary complications ($n = 15$), gastrointestinal bleeding ($n = 6$) or other reasons ($n = 6$).

Diagnosis of IA

Twenty-five patients developed proven ($n = 8$) or probable ($n = 17$) IA at a median of 204 days (range 21–1527 days) after HSCT, with a 1 year cumulative incidence of 12.9% (Figure 1). Twenty-two patients (88%) had pulmonary disease, two of whom showed dissemination. The remaining three had tracheobronchitis, sinusitis and gastrointestinal involvement, respectively. IA was the direct cause of death in five patients. Positive GM antigenaemia was observed in 22 patients with proven or probable IA. In a patient-based analysis, the sensitivity and specificity of the test were 88% (22 of 25) and 79% (104 of 132), respectively.

Episodes with positive GM antigenaemia

A total of 3296 serum samples were analysed from 157 patients (mean, 21 samples/patient; range, 2–109 samples/patient). Overall, 50 patients (31.9%) developed positive GM antigenaemia at a median of 107 days (range 12–1193 days) after HSCT, with a 1 year cumulative incidence of 32.2% (Figure 1). Five patients had second positive episodes at a median interval of 358 days (range 119–1103 days) between the first and second episodes. Four positive episodes occurred in one patient.

A total of 58 positive episodes of the 50 patients were therefore analysed (Table 2). Twenty-two episodes were diagnosed true-positive based on the diagnosis of proven or probable IA. In these patients, the microbiological criterion was fulfilled with pathological findings and/or culture results in 10 and GM antigen test in 12. Seven were considered inconclusive-positive. In all the seven episodes, we could not conclude whether the abnormalities on CT scans were attributed to IA or not, because antifungal agents were administered empirically ($n = 5$) or for the treatment of documented candidiasis ($n = 2$) at the time of positive GM antigenaemia.

Twenty-nine episodes were considered false-positive, in all of which piperacillin/tazobactam or amoxicillin/clavulanate was not given at the time of positive GM antigenaemia. *Penicillium* and

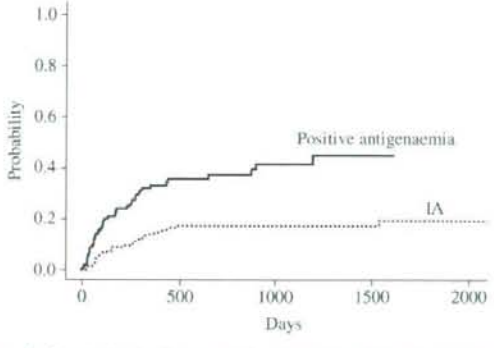

Figure 1. Cumulative incidences of IA and positive GM antigenaemia after HSCT.

Table 2. Incidence of false-positive GM antigenaemia

	Total episodes	Episodes before day 100	Episodes after day 100
True-positive	22	8	14
False-positive	29	15	14
Inconclusive-positive	7	1	6
Total	58	24	34
False-positive rate (%)	50	62.5	41.2

Paecilomyces were not detected in these false-positive episodes. At the time of false-positive antigenaemia, antifungal prophylaxis was given in 23 episodes (fluconazole, 20; itraconazole, 3), and no antifungal agents at all in the remaining 6. Empirical or targeted antifungal therapy was not performed in these episodes. CT scans were performed in 22 episodes, in which no radiological abnormalities were seen in 12, and non-specific abnormalities in the remaining 10 were caused by *P. jirovecii* infections ($n = 2$), bacterial infections ($n = 2$), pulmonary involvement of cancer ($n = 1$), heart failure ($n = 1$), bronchiolitis obliterans organizing pneumonia (BOOP) ($n = 1$) or unknown aetiology ($n = 3$). All three unexplained radiological abnormalities disappeared spontaneously.

Incidence and risk factors for false-positive GM antigenaemia

Of the 58 positive episodes, 29 satisfied the criteria of false-positive antigenaemia, with a false-positive rate of 50% (Table 2). During the first 100 days after HSCT, 15 of 24 positive episodes were considered false-positive, with a false-positive rate of 62.5% (Table 2). PPV was 33.3% or 37.5% when we included the inconclusive episode into the false-positive group or the true-positive group, respectively, in the 24 positive episodes. PPV was 55.6% or 66.7% even in nine with grade II–IV acute GVHD at the time of positive GM antigenaemia. In contrast, 14 of 34 positive episodes beyond 100 days were considered false-positive, with a rate of 41.2%, and PPV was 41.2% or 58.8%. False-positive antigenaemia occurred more frequently and therefore PPV was lower during the first 100 days.

There were no significant parameters that increased the incidence of false-positive GM antigenaemia over the entire period and during the first 100 days (Tables 3 and 4). The incidence was rather decreased in the presence of active GVHD (at any grade) and liver GVHD over the entire period, and grade II–IV GVHD, grade III–IV GVHD and liver GVHD during the first 100 days. In contrast, gastrointestinal chronic GVHD was identified as the only significant risk factor for increased false-positive GM antigenaemia beyond 100 days (Table 5). Twenty of the 30 episodes of positive GM antigenaemia without gastrointestinal chronic GVHD were true-positive, whereas all 4 positive GM antigenaemia episodes in patients with gastrointestinal chronic GVHD were false-positive (PPV 66.7% versus 0%, $P = 0.02$). Gastrointestinal chronic GVHD in these patients was associated with more than 500 mL of diarrhoea at the time of positive GM antigenaemia, the diagnosis of which was pathologically confirmed with colon biopsy.

Table 3. Risk factors for false-positive GM antigenaemia after HSCT

Factors	False-positive	Others	*P* value
Age			
>40 years	18	18	1.00
≤40 years	11	11	
Disease risk			
standard risk	7	5	0.75
high risk	22	24	
Graft source			
bone marrow	16	15	0.79
peripheral blood	13	14	
Donor type			
matched sibling donor	9	9	1.00
alternative donor	20	20	
Neutrophil count			
<500 cells/μL	2	3	1.00
≥500 cells/μL	27	26	
Active GVHD on positive GM			
yes	13	23	0.01
no	16	6	
Gastrointestinal GVHD on positive GM			
yes	6	3	0.47
no	23	26	
Liver GVHD on positive GM			
yes	5	14	0.02
no	24	15	
Skin GVHD on positive GM			
yes	137	20	0.41
no	105	50	
Prednisolone on positive GM (1)			
≥0.5 mg/kg	137	20	0.41
<0.5 mg/kg	105	50	
Prednisolone on positive GM (2)			
≥1.0 mg/kg	137	20	1.00
<1.0 mg/kg	105	50	

In thorough examinations for aspergillosis, no radiological abnormalities were seen in two patients, non-specific abnormalities on CT scan were observed but spontaneously disappeared without clinical symptoms suggestive of IA in one, and radiological findings compatible with BOOP were observed and promptly improved with systemic corticosteroids in one. There was another false-positive episode probably associated with gastrointestinal chronic GVHD, which was included in the 'no gastrointestinal chronic GVHD' group because GVHD was absent at the detection of positive GM antigenaemia, but gastrointestinal chronic GVHD developed soon thereafter. Among these five episodes, the GM levels became normal with the improvement of gastrointestinal chronic GVHD in four, whereas GM antigen monitoring was discontinued because of death from haematological relapse in the remaining one.

Table 4. Risk factors for false-positive GM antigenaemia before day 100

Factors	False-positive	Others	*P* value
Neutrophil count			
<500	1	1	1.00
≥500	14	8	
Active GVHD on positive GM			
yes	4	6	0.09
no	11	3	
Grade II–IV acute GVHD on positive GM			
yes	3	6	0.04
no	12	3	
Grade III–IV acute GVHD on positive GM			
yes	0	3	0.04
no	15	6	
Gastrointestinal GVHD on positive GM			
yes	2	3	0.33
no	13	6	
Liver GVHD on positive GM			
yes	0	5	<0.01
no	15	4	
Skin GVHD on positive GM			
yes	3	4	0.36
no	12	5	
Prednisolone on positive GM (1)			
≥0.5 mg/kg	9	5	1.00
<0.5 mg/kg	6	4	
Prednisolone on positive GM (2)			
≥1.0 mg/kg	5	4	0.68
<1.0 mg/kg	10	5	

Table 5. Risk factors for false-positive GM antigenaemia after day 100

Factors	False-positive	Others	*P* value
Active GVHD on positive GM			
yes	9	17	0.23
no	5	3	
Extensive chronic GVHD on positive GM			
yes	7	10	1.00
no	7	10	
Gastrointestinal GVHD on positive GM			
yes	4	0	0.02
no	10	20	
Liver GVHD on positive GM			
yes	5	9	0.73
no	9	11	
Skin GVHD on positive GM			
yes	5	8	1.00
no	9	12	
Oral GVHD on positive GM			
yes	3	6	0.70
no	11	14	
Prednisolone on positive GM (1)			
≥0.5 mg/kg	3	3	0.67
<0.5 mg/kg	11	17	
Prednisolone on positive GM (2)			
≥1.0 mg/kg	2	2	1.00
<1.0 mg/kg	12	18	

Discussion

This study demonstrated that the sensitivity of the GM ELISA test was 88% in patient-based analysis and PPV was 38% to 50% in episode-based analysis, which were comparable with those in previous reports.[3–6] However, false-positive GM antigenaemia frequently occurred during the first 100 days after HSCT, and PPV was lower even among patients with grade II–IV acute GVHD, in whom the pre-test probability of IA was considered to be much higher than patients without acute GVHD.

A significant correlation between the occurrence of false-positive GM antigenaemia and the presence of gastrointestinal chronic GVHD was observed in this study. GM ELISA results were false-positive in all four episodes with gastrointestinal chronic GVHD at the time of positive GM antigenaemia, and there was another false-positive episode in which GVHD was absent at the detection of positive GM antigenaemia, but gastrointestinal chronic GVHD developed soon thereafter. During these episodes, piperacillin/tazobactam or amoxicillin/clavulanate was not given, and occult infections by some fungi reacting with GM ELISA were not detected, both of which were previously reported as important risk factors for false-positive GM antigenaemia.[11–13] Meanwhile, our results were consistent with the conclusions of other studies that concurrent mucositis in HSCT recipients or immature intestinal mucosa in neonates allows the translocation of GM contained in foods, leading to frequent false-positive GM antigenaemia.[3–5,8–10] These findings suggested the possibility that passage of dietary GM into the blood from the disrupted intestinal mucosal barrier might result in false-positive antigenaemia in patients with gastrointestinal chronic GVHD.

In contrast, the development of gastrointestinal acute GVHD was not significantly associated with the occurrence of false-positive GM antigenaemia in our series. This was probably because the overall false-positive rate during the first 100 days after HSCT was higher than that beyond 100 days. Mucosal damage due to the high-dose chemotherapy or TBI in the conditioning regimen might be the cause of frequent false-positive GM antigenaemia early after HSCT.[5]

Pfeiffer *et al.*[19] recently showed the significant heterogeneity of GM test performance among patients with different prevalences of IA. They demonstrated that GM assay was more useful in immunocompromised high-risk populations such as HSCT recipients or patients with haematological malignancy than in solid-organ transplant recipients. Although emphasizing the utility of GM assay only when there is a high pre-test probability of IA, they also addressed the need for further investigations of the reasons for the heterogeneity. Prior antifungal therapy and false-positive results are possible explanations for the heterogeneity, and our findings may contribute to the effective use of the assay. However, our study is a retrospective evaluation and therefore there are some potential weaknesses. In this study,

regular screening of GM antigen was not rigorously performed, but on an on-demand basis. This is in contrast to the previous studies in which GM antigenaemia was evaluated more intensively.[3–5] This fact might have affected the diagnostic performance of this assay, but the high cost of this test precluded such intensive monitoring in daily practice. In addition, we should mention that this study might lack enough statistical power to detect the other risk factors for false-positive antigenaemia than gastrointestinal chronic GVHD because of the small number of patients with positive antigenaemia. Also, the small number of patients with positive antigenaemia precludes multivariate analysis, which might be another reason for failing to find the possible impact of the other risk factors. The other major limitation is that GM antigenaemia itself was included in the microbiological criteria, which might have precluded the evaluation of true performance of this assay. In this study, however, the number of patients diagnosed with IA falls from 22 to 10, if the GM results are excluded from the criteria, which seemed too small for the statistical analysis. Therefore, we used the original EORTC/MSG definitions that include GM antigenaemia in the microbiological criteria.

In conclusion, frequent false-positive GM antigenaemia was observed in allo-HSCT recipients during the first 100 days after transplantation or in those with gastrointestinal chronic GVHD, leading to a decreased PPV of the GM ELISA test. Therefore, GM antigenaemia results should be considered cautiously in these patients in conjunction with other diagnostic procedures including CT scans.

Acknowledgements

We thank all the clinicians who have assisted with the provision of data for this project.

Funding

This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare.

Transparency declarations

None to declare.

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Long-term ultra-low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation

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To evaluate the efficacy of long-term prophylaxis with ultra-low-dose acyclovir against varicella-zoster virus (VZV) reactivation, we analyzed the records of 242 Japanese adult patients who underwent allogeneic hematopoietic stem cell transplantation for the first time from 1995 to 2006 at our hospital. We started long-term oral acyclovir at 200 mg/day in July 2001. Acyclovir was continued until the end of immunosuppressive therapy and at least 1 year after transplantation. Sixty-six patients developed VZV reactivation at a median of 248 days after HSCT, with a cumulative incidence of 34.7%. Only one breakthrough reactivation occurred during long-term acyclovir, which responded well to therapeutic dose of valacyclovir. The use of long-term acyclovir was the only independent determinant that significantly decreased the overall incidence of VZV reactivation (20% vs. 50%, $P < 0.0001$). With this prophylaxis, visceral dissemination and serious complications other than post-herpetic neuralgia was completely eliminated, and thereby need for hospitalization was significantly reduced (21% vs. 71%, $P = 0.0034$). Fifteen of the 57 patients who discontinued acyclovir developed VZV reactivation, with a cumulative incidence of 32.1%. VZV reactivation following discontinuation tended to occur in patients who were receiving immunosuppressive therapy at the cessation of acyclovir. These findings suggested that long-term prophylaxis of ultra-low-dose acyclovir resulted in a successful prevention of severe VZV-related symptoms and death, with a significantly decreased overall incidence of VZV reactivation. Prolongation of prophylactic acyclovir on profound immunosuppression might be important for thorough suppression of VZV reactivation. Am. J. Hematol. 83:472–476, 2008. © **2008 Wiley-Liss, Inc.**

Introduction

Varicella-zoster virus (VZV) infection remains a common complication after hematopoietic stem cell transplantation (HSCT) [1–4]. VZV infection develops as a reactivation of latent virus mainly between the third and twelfth month after transplantation, with a cumulative incidence of more than 30% [1,2]. Localized dermatomal rash is the most common clinical presentation, whereas dissemination or visceral involvement is occasionally observed, leading to a fatal outcome. Although most of VZV infections were successfully treated with antiviral agents, VZV-related complications including post-herpetic neuralgia and secondary infection significantly affect the patient's quality of life [1,5].

The introduction of long-term prophylaxis with low-dose acyclovir against VZV reactivation has therefore been investigated [4,6–10]. Several studies concluded that prophylactic acyclovir at 600–3,200 mg/day continued for a fixed period up to 6 months or 1 year have failed to decrease the overall incidence of VZV reactivation [4,6–8]. Despite that VZV reactivation during prophylaxis was significantly reduced, a substantial number of VZV reactivation occurred following the discontinuation of acyclovir. A most recent randomized placebo-controlled trial showed a predominant occurrence of VZV reactivation after the cessation of acyclovir, which was given at 800 mg/day for 1 year after HSCT, in recipients with prolonged immunosuppression [8]. Moreover, other studies reported that long-term acyclovir at 400 mg/day continued until the end of immunosuppressive therapy could not suppress VZV reactivation after the discontinuation of acyclovir [9,10]. Thus, the appropriate prophylactic dose and duration of acyclovir to decrease the overall incidence of VZV reactivation have not been clarified.

We carried out a novel trial of long-term acyclovir prophylaxis at an ultra-low-dose (200 mg/day) until the end of immunosuppressive therapy and at least 1 year after HSCT, and retrospectively compared the incidence of VZV reactivation with historical control patients who did not receive long-term prophylaxis. With this prophylaxis, lower-cost, less side effects, and better compliance may also be promising.

Results

Incidence and risk factors for VZV reactivation after HSCT

In total of 242 patients, 137 received long-term acyclovir following prophylaxis against HSV infection, whereas the remaining 105 did not receive long-term acyclovir. Overall, 66 out of the 242 patients developed VZV reactivation at a median of 248 days (range 50–1,494 days) after HSCT,

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Contract grant sponsor: Ministry of Health, Labor and Welfare.

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Received for publication 17 July 2007; Revised 14 December 2007; Accepted 27 December 2007

Am. J. Hematol. 83:472–476, 2008.

Published online 10 January 2008 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21152

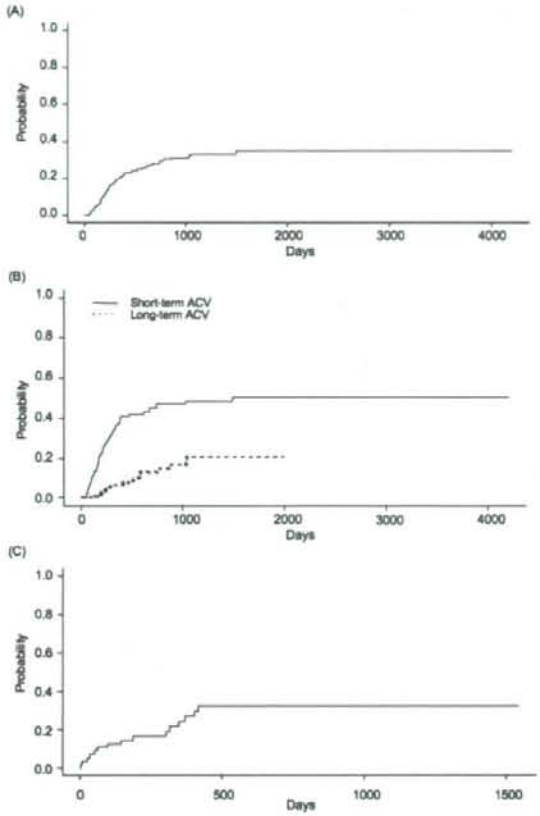

Figure 1. (A) Cumulative incidences of VZV reactivation after HSCT in all 242 patients. (B) Cumulative incidences of VZV reactivation after HSCT in 137 patients who received long-term acyclovir versus 105 patients who did not. (C) Cumulative incidences of VZV reactivation after the cessation of long-term acyclovir in 57 eligible patients for analysis.

TABLE I. Risk Factors for VZV Reactivation After HSCT

Factors	Variables	n	Incidence (%)	*P*-value
Univariate analysis				
Age	≥ 40 years old	117	25	0.005
	<40 years old	125	43	
Sex	Male	154	34	0.71
	Female	88	37	
Disease risk	Standard-risk	96	38	0.63
	High-risk	146	32	
Graft source	Bone marrow	166	40	0.06
	Peripheral blood	73	25	
Donor type	Matched sibling donor	97	40	0.11
	Alternative donor	145	31	
Regimen (1)	Conventional	204	37	0.05
	Reduced-intensity	38	25	
Regimen (2)	TBI regimen	179	36	0.63
	Non-TBI regimen	63	32	
Long-term ACV	Yes	137	20	<0.0001
	No	105	50	

Factors	Variables	*n*	Relative risk 95% CI	*P*-value
Grade II–IV acute GVHD	Yes	97	1.18 (0.72–1.94)	0.51
	No	145		
Chronic GVHD	Yes	131	0.87 (0.51–1.50)	0.62
	No	76		

Factors	Relative risk	95% CI	*P*-value
Multivariate analysis			
Long-term ACV	0.23	0.13–0.39	<0.0001

with a cumulative incidence of 34.7% (Fig. 1A). Only one patient experienced a breakthrough reactivation during long-term acyclovir, which responded promptly to a therapeutic dose of valacyclovir. In univariate analyses, younger age, bone marrow transplantation, conventional regimen, and the use of long-term acyclovir were significantly associated with the low VZV reactivation incidence rate (Table I). In a multivariate analysis, the use of long-term acyclovir was identified as the only independent factor that significantly decreased the incidence of VZV reactivation (20% vs. 50%, $P < 0.0001$, Table I, Fig. 1B).

Clinical features of patients who developed VZV reactivation

Fifty-three of the 66 VZV reactivations (80%) occurred in a localized dermatomal distribution (Table II). Clinically significant complications developed in 17 patients, the most common of which was post-herpetic neuralgia. Among these complications, only post-herpetic neuralgia was seen in three patients with long-term acyclovir, whereas serious complications including CNS involvement, motor neuropathy, and ophthalmic complications were involved in the remaining 14 patients without long-term acyclovir.

Fifty-two of the 66 patients developed VZV reactivation in outpatient setting. Among these patients, hospitalization was required for VZV reactivation in 3 of 14 patients with long-term acyclovir and in 27 of 38 patients without long-term acyclovir (21% vs. 71%, $P = 0.0034$).

Seven of the 66 patients with VZV reactivation (11%) developed recurrent VZV reactivation in the different dermatome, at a median of 95 days (range 55–798 days) after the first episode. All of them never received acyclovir after finishing the treatment for the first episode. At the time of recurrence, five of the seven patients were receiving immunosuppressive therapy and the remaining two showed severe lymphocytopenia less than 300/μl due to chemotherapy for relapse of hematological malignancy. The third episode of VZV reactivation occurred in two patients, at 158 and 240 days after the second reactivation. None was receiving acyclovir at the time of second or third VZV reactivation. All the patients responded well to treatment with antiviral agents, and none of them directly died of VZV reactivation.

Incidence and risk factors for VZV reactivation after the cessation of long-term acyclovir

Of 137 patients who received long-term acyclovir, 73 patients were receiving acyclovir until VZV reactivation, their last follow-up, or death. The other seven died within a week following the discontinuation of acyclovir. Therefore, 80 patients were excluded and only 57 patients were eligible for analysis after the cessation of acyclovir. The median follow-up duration from the discontinuation of acyclovir was 279 days (range 9–1,936 days). They received long-term acyclovir with a median prophylactic period of 358 days (range 49–1,259 days). Fifteen patients developed VZV reactivation at a median of 147 days (range 5–415 days)

TABLE II. Clinical Presentation and Secondary Complications of VZV Reactivation

Low-dose ACV	No	Yes	Total
Total patients	105	137	242
VZV reactivation	50	16	66
Out-patient onset	38	14	52
Hospitalized	27	3	30
Treated as outpatient	11	11	22
Valacyclovir	4	8	12
Acyclovir	7	3	10
Clinical presentations			
Localized	39	14	53
Trigeminal	4	2	6
Cervical	5	1	6
Thoracic	22	5	27
Lumbar	5	4	9
Sacral	3	2	5
Disseminated	11	2	13
Cutaneous	7	2	9
Visceral	4	0	4
Complications	14	3	17
Ophthalmic complications	1	0	1
Motor neuropathy	1	0	1
CNS involvement	3*	0	3
Post-herptic neuralgia	9	3	12

*One patient had both CNS involvement and post-herpetic neuralgia.

TABLE III. Risk Factors for VZV Reactivation After the Cessation of Long-Term ACV

Factors	Variables	*n*	Incidence(%)	*P*-value
Univariate analysis				
Age	≥40 years old	31	34	0.56
	<40 years old	26	29	
Sex	Male	36	21	0.14
	Female	21	54	
Disease risk	Standard-risk	26	30	0.39
	High-risk	31	34	
Graft source	Bone marrow	28	34	0.96
	Peripheral blood	28	41	
Donor type	Matched sibling donor	23	29	0.61
	Alternative donor	34	34	
Regimen (1)	Conventional	43	32	0.94
	Reduced-intensity	14	31	
Regimen (2)	TBI regimen	42	30	0.57
	Non-TBI regimen	15	38	
Duration of long-term ACV	<1 year	33	30	0.85
	≥1 year	24	33	
Immunosuppressive therapy at the cessation of ACV	Yes	25	44	0.12
	No	32	20	

Factors	Variables	*n*	Relative risk 95% CI	*P*-value
Chronic GVHD	Yes	37	1.68	0.47
	No	17	(0.40–6.99)	

after the discontinuation of acyclovir, with a cumulative incidence of 32.1% (Fig. 1C). Although statistically significant risk factors were not identified to affect the incidence of VZV reactivation after discontinuation, ongoing immunosuppressive therapy at the cessation of acyclovir tended to increase the incidence of VZV reactivation (Table III).

Discussion

This study demonstrated that the long-term prophylactic acyclovir at 200 mg/day was highly effective to reduce VZV reactivation, dissemination and serious complications, as well as VZV-related mortality in HSCT recipients. There was only one breakthrough of localized reactivation that responded well to the therapeutic dose of valacyclovir. A once-a-day dosing of 200 mg until the cessation of immunosuppressive therapy and at least 1 year after HSCT significantly decreased the overall incidence of VZV reactivation from 50 to 20%, in contrast with the previous studies in which various doses of 600 mg/day or more were given for a fixed period up to 6 months or 1 year after HSCT without significant reduction of the overall incidence of VZV reactivation [4,6–8]. Although an optimal prophylactic dose and duration of acyclovir administration has not been clarified, this extended prophylactic approach to continue acyclovir until the end of immunosuppressive therapy and at least 1 year after HSCT may be more appropriate than the shorter prophylaxis or fixed-duration prophylaxis. Also, this is the first report that the ultra-low-dose of acyclovir at only 200 mg/day was sufficient to prevent VZV reactivation during prophylaxis.

In this study, however, VZV reactivation was not uncommon after the discontinuation of long-term acyclovir, as previously observed in the other two studies in which acyclovir at 400 mg/day was given until the end of immunosuppressive therapy [9,10]. Nevertheless, the severity of clinical symptoms was ameliorated and thereby need for hospitalization was markedly reduced by the long-term acyclovir. Among the 15 patients who developed VZV reactivation after the cessation of acyclovir, none showed visceral dissemination or serious complications. The less severe symptoms in patients with long-term acyclovir may reflect the contribution of VZV-specific immune recovery, which might have been accelerated by subclinical VZV reactivation. It has been shown that *in vivo* re-exposure to VZV antigens without clinical symptoms may boost immunity and thereby prevent subsequent symptomatic VZV reactivation [11]. Lower daily dosing of 200 mg might have permitted subclinical VZV reactivation to establish the reconstitution of VZV-specific immunity. There is another possibility that need for hospitalization in patients with long-term acyclovir might have been reduced by the use of valacyclovir, which became available from October 2000 in Japan. However, mild cases of VZV reactivation had been treated with oral acyclovir, and actually 7 of 11 patients who developed VZV reactivation without long-term acyclovir were successfully treated with oral acyclovir without hospitalization. Therefore, we suppose that a decreased hospitalization rate in patients with long-term acyclovir was due to less severe symptoms rather than the availability of valacyclovir.

In some patients, long-term acyclovir was discontinued within a year at the physician's discretion or at the request of the patients. This is a limitation of this study, but it revealed that ongoing immunosuppressive therapy at the cessation of acyclovir tended to be more frequently associated with VZV reactivation following discontinuation, which agreed with the conclusion of Boeckh's study that VZV reactivation predominantly occurred in patients with continued systemic immunosuppression [8]. They did not find any significant difference in the reconstitution of VZV-specific immunity between the acyclovir and placebo groups following the 1-year prophylaxis at 800 mg/day. In addition, the study with long-term acyclovir at 400 mg/day also showed that VZV reactivation after the cessation of acyclovir was observed only in patients who were receiving resumed immunosuppressants [10]. In this study, three patients with long-term acyclovir experienced dissemination and/or post-

herpetic neuralgia, all of whom were receiving prolonged immunosuppressive therapy for chronic GVHD both at the cessation of acyclovir and at the time of VZV reactivation. These findings suggest that VZV reactivation as well as the severity of symptoms is strongly related to the decline in VZV-specific immunity as a result of HSCT and/or immunosuppressive therapy. Therefore, continuing acyclovir in patients with profound immunosuppression is recommended for further prevention of VZV reactivation. Another possible approach is to administer inactivated VZV vaccine at the discontinuation of acyclovir [12].

In conclusion, this study showed that the long-term prophylaxis with ultra-low-dose acyclovir might be an effective strategy for the suppression of VZV reactivation during prophylaxis and minimizing the long-term risks of VZV-related complications and mortality. Further investigation is necessary to evaluate the validity of resuming acyclovir for patients with resumed immunosuppressive therapy.

Patients and Methods

Study population

A total of 271 consecutive adult patients (≥16 years old) underwent allogeneic HSCT for the first time at the University of Tokyo Hospital between June 1995 and November 2006. Five patients who died within 35 days after HSCT were excluded, and clinical data for this study were available for 242 of the remaining 266 patients. A median follow-up was 486 days (range, 37–4,209 days) from HSCT for the entire cohort of 242 patients. Thirty-eight patients who received reduced-intensity conditioning were included. The patient characteristics are summarized in TABLE IV. Ninety-seven, 42 and 103 patients received graft from an HLA-matched sibling donor, a mismatched related donor, and a matched unrelated donor, respectively. Unrelated HSCT was performed exclusively using bone marrow, whereas 73 out of 139 related donors chose to donate peripheral blood stem cell graft. Acute leukemia in first remission, chronic myelogenous leukemia in first chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, and aplastic anemia were defined as low-risk diseases, while others were considered high-risk diseases. Donors other than HLA-matched sibling donors were defined as alternative donors.

Transplantation procedure

The conventional preparative regimen for leukemia/lymphoma was mainly performed with either total body irradiation (TBI) regimen (cyclophosphamide (Cy) at 60 mg/kg for 2 days and TBI at 2 Gy twice daily for 3 days) or non-TBI regimen (Cy at the same dose combined with busulfan (Bu) at 4 mg/kg for 4 days). In TBI regimen, the dose of Cy was decreased to 40 mg/kg for 1 day and etoposide at 20 mg/kg for 2 days was added instead in patients with impaired cardiac function. Fludarabine (Flu)-based regimens included FB regimen (Flu at 30 mg/m^2 for 6 days and Bu at 4 mg/kg for 2 days) with or without TBI at 4 Gy, FB16 regimen (Flu at the same dose with Bu at 4 mg/kg for 4 days), FM regimen (Flu 30 mg/m^2 for 5 days and melphalan at 140 mg/m^2 for 1 day), and FC regimen (Flu at 25 mg/m^2 for 5 days and Cy at 60 mg/kg for 2 days) were used as reduced-intensity regimens for elderly or clinically infirm patients [13]. Gemcitabine at 1,000 mg/kg/m^2 for 3 days was added to the FB regimen for patients with pancreatic cancer [14]. The conditioning regimen for aplastic anemia was either a rabbit antithymocyte globulin (ATG) regimen (Cy at 50 mg/kg for 4 days and ATG at 5 mg/kg for 5 days with or without TBI at 4 Gy) or an alemtuzumab regimen (Cy at 25 mg/kg for 4 days and Flu at 30 mg/kg for 4 days combined with alemtuzumab at 0.2 mg/kg for 6 days, with or without TBI at 2 Gy).

For prophylaxis against GVHD, cyclosporine A (CsA) at 3 mg/kg/day or tacrolimus at 0.03 mg/kg/day was combined with short-term methotrexate (10–15 mg/m^2 on Day 1, 7–10 mg/m^2 on Days 3 and 6, and optionally on Day 11). For patients who received graft from an HLA-mismatched donor, alemtuzumab was added to the TBI regimen or the FB regimen at 0.2 mg/kg for 6 days [15]. Methyl-prednisolone (mPSL) or prednisolone (PSL) at 1 or 2 mg/kg was added for patients who developed grade II-IV acute GVHD, whereas PSL at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, fungal, and *Pneumocystis jirovecii* infections consisted of fluconazole, tosufloxacin, and sulfamethoxazole/trimethoprim. Antigenemia-guided pre-emptive therapy against CMV infection was performed as described previously [16].

TABLE IV. Patients' Characteristics

Characteristic		Total patients
Sex (male/female)		154/88
Age, median (range)		39 (16–66)
Underlying disease	Acute leukemia	121
	CML	50
	MDS	26
	NHL/ATL	25
	SAA	10
	Other	10
Graft source	PBSC	73
	BM	166
	CB	3
Donor type	Matched sibling	97
	Mismatched related	42
	Unrelated	103
VZV seropositivity	Positive	231
	Negative	3
	Not examined	8
Preparative regimen	Cy (Etp)/TBI-based regimens	167
	Bu/Cy-based regimens	37
	ATG-based regimens for SAA	7
	Flu-based RIC	31
GVHD prophylaxis	CsA + MTX	200
	Tacrolimus + MTX	18
	Alemtuzumab + CsA + MTX	24
Acute GVHD	Grade 0–I	145
	Grade II–IV	97
Chronic GVHD	Extensive	86
	Limited	45
	None	76

VZV indicates varicella zoster virus; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; ATL, adult T-cell leukemia/lymphoma; SAA, severe aplastic anemia; PBSC, peripheral blood stem cell; BM, bone marrow; CB, cord blood; Cy, cyclophosphamide; Etp, etoposide; TBI, total body irradiation; Bu, busulfan; ATG, antihymocyte globulin; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate.

Diagnosis and treatment of VZV reactivation

The diagnosis of VZV reactivation was established by the presence of characteristic vesicular skin lesion on an erythematous base within dermatome or a generalized cutaneous distribution. Microbiological and/or pathological confirmation was performed only in equivocal cases. Post-herpetic neuralgia was defined as dermatomal pain persisting beyond 1 month after initial presentation of VZV reactivation. VZV reactivation was treated with intravenous acyclovir at 15–30 mg/kg/day in the majority of patients, and followed, in some patients, by oral acyclovir at 1–4 g/day or oral valacyclovir at 3 g/day, for a total treatment period of 5–42 days. A proportion of patients received outpatient treatment only, with valacyclovir 3 g/day orally for 5–10 days. The doses and dosing interval of these drugs were adjusted according to the creatinine clearance in patients with renal impairment.

Prophylactic administration of acyclovir

As prophylaxis against herpes simplex virus infection (HSV), acyclovir was given at 750 mg/day intravenously or at 1,000 mg/day orally from Day 7 to 35. We started long-term oral administration of acyclovir at an ultra-low-dose (200 mg/day) as prophylaxis against VZV reactivation (hereinafter described as "long-term acyclovir") in July 2001, and it was applied for all allogeneic transplantation recipients thereafter. Long-term acyclovir was principally given from Day 36 until the end of immunosuppressive therapy and at least 1 year after HSCT. When intravenous ganciclovir was required for the treatment of CMV infection, acyclovir was discontinued during the course of intravenous ganciclovir

and resumed afterward. In some patients, acyclovir was discontinued within a year or before the cessation of immunosuppressive therapy at the physician's discretion or at the request of patients themselves.

Statistical analysis

The cumulative incidence of VZV reactivation and the impact of possible confounding factors on VZV reactivation were evaluated using Gray's method, considering death without VZV reactivation as a competing risk [17]. The development of acute and chronic GVHD was treated as time-dependent covariates. The influence of chronic GVHD was evaluated only in patients who survived longer than 100 days. Factors associated with at least borderline significance ($P < 0.10$) in univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. *P*-values of less than 0.05 were considered statistically significant.

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Journal of Medical Virology 80:1769–1775 (2008)

Case Report: Persistent Cytomegalovirus (CMV) Infection After Haploidentical Hematopoietic Stem Cell Transplantation Using In Vivo Alemtuzumab: Emergence of Resistant CMV Due to Mutations in the UL97 and UL54 Genes

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Addition of in vivo alemtuzumab to the conditioning regimen enabled 2- or 3-locus-mismatched hematopoietic stem cell transplantation with an acceptable incidence of graft-versus-host-disease. However, the procedure was associated with a high incidence of cytomegalovirus (CMV) reactivation. Although preemptive therapy with ganciclovir prevented successfully severe CMV diseases and CMV-related mortality, a patient developed persistent positive CMV antigenemia for more than 1 year after transplantation and CMV disease, despite the use of ganciclovir and foscarnet. The in vitro susceptibility assay showed that the clinical isolate was resistant to foscarnet, moderately resistant to ganciclovir, but sensitive to cidofovir. Therefore, cidofovir was administered. CMV antigenemia became negative within 2 weeks and never developed again. Nucleotide sequence of the UL54 and UL97 of the clinical isolate showed 4 amino acid substitutions (V11L, Q578H, S655L, and G874R) in UL54 and 2 mutations (A140V and A594V) in UL97 compared with the Towne and AD169 strains. Ganciclovir resistance was suspected to be caused by both A594V of UL97 and Q578H of UL54, whereas foscarnet resistance was due mainly to Q578H of UL54. In conclusion, the in vitro susceptibility assay as well as nucleotide sequence of clinical isolate is important to choose appropriate antiviral agents for patients who have persistent CMV reactivation after stem cell transplantation. ***J. Med. Virol. 80:1769–1775, 2008.***

KEY WORDS: CMV; resistance; mutation; hematopoietic stem cell transplantation; alemtuzumab

INTRODUCTION

Cytomegalovirus (CMV) disease is one of the major complications after allogeneic hematopoietic stem cell transplantation. Since the mortality rate is high, preemptive strategy by monitoring CMV reactivation with antigenemia assay or DNAemia assessed by the polymerase chain reaction have been developed for the management of CMV after hematopoietic stem cell transplantation [Boeckh et al., 1996; Kanda et al., 2002]. The risk factors for CMV disease after allogeneic hematopoietic stem cell transplantation include transplantation from an unrelated donor, the presence of HLA mismatch, the use of T-cell depletion, the development of acute graft-versus-host disease, the use of steroids, and so on [Nichols et al., 2001; Kanda et al., 2001a; Asano-Mori et al., 2005].

Alemtuzumab (Campath-1H) is a humanized monoclonal antibody directed against human CD52, that is

Grant sponsor: Ministry of Health, Labor, and Welfare; Grant number: H18-Shinkou-013.

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Accepted 20 May 2008

DOI 10.1002/jmv.21277

Published online in Wiley InterScience (www.interscience.wiley.com)

expressed at a high density on B and T lymphocytes and dendritic cells, but not on hematopoietic stem cells [Gilleece and Dexter, 1993]. Therefore, the addition of alemtuzumab to the pretransplant conditioning regimen results in in vivo T-cell depletion and prevents acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation, even from HLA-mismatched donors [Kottaridis et al., 2000; Chakraverty et al., 2002; Kanda et al., 2005]. Although the use of alemtuzumab for in vivo T-cell depletion has been also reported to be a risk factor for CMV reactivation, preemptive therapy with ganciclovir or foscarnet can prevent an excessive incidence of CMV diseases and CMV-related mortality [Bainton et al., 2002; Nguyen et al., 2002; Laurenti et al., 2004; Kanda et al., 2005]. However, one patient developed persistent CMV antigenemia more than 1 year after haploidentical HLA-mismatched hematopoietic stem cell transplantation using in vivo alemtuzumab despite the use of ganciclovir and foscarnet. The clinical course of the patient and the results of the in vitro susceptibility assay and nucleotide sequence of the clinical isolate are described.

MATERIALS AND METHODS

Plaque Reduction Assay

The susceptibilities of the CMV isolate to ganciclovir, cidofovir, and foscarnet were examined by the plaque reduction assay [Cockley et al., 1988; Shiraki et al., 1990, 1991a,b; Yukawa et al., 1996]. Briefly, all assays were carried out in confluent HEL cell monolayers in 60 mm plastic dishes. The cells were infected with 100 plaque forming units (PFU)/0.2 ml of the CMV isolate and the Towne strain for 1 hr. Then, they were exposed to various concentrations of the drugs in 0.8% nutrient methylcellulose minimum essential medium supplemented with 2% fetal bovine serum, and incubated at 37°C as indicated below. The infected cells were fixed with neutral formalin and stained with methylene blue and then the number of plaques was counted. The inhibitory concentrations for 50% plaque reduction (IC_{50}) values were determined using the graph software MPM III-vs. 1.57 for Macintosh.

Sequence Determination of the UL57 and UL97 Genes

CMV DNA was prepared from the viral nucleocapsid as described previously [Shiraki et al., 1991; Ida et al., 1999; Yoshida et al., 2005]. The UL57 (DNA polymerase) and UL97 (phosphotransferase) genes were amplified by the primer sets of 5′-CCAACGAGCAGGCTTACC-3′ and 5′GTCGTCCTACGCGCGATACG-3′, and 5′-ACGCCTCTGTTCAGATTTTA-3′ and 5′-CCCACATGTAGATGGCGGCG-3′, respectively. The amplified fragments were sequenced by using ABI PRISM 3100 DNA sequencer according to the manufacture's procedures. The determined CMV UL54 and UL97 sequences were compared with the Towne (D14980 and U07355) and AD169 (X17403) strains and the nucleotide differences of the isolate common to the both were identified as the significant nucleotide changes.

Nucleotide Sequence Accession Numbers

The nucleotide sequences determined in this study have been deposited in the GenBank/DDBJ/EMBL database. The accession numbers of the CMV UL54 and UL97 genes from the patient were AB329634 and AB329635, respectively. The accession numbers of UL54 and UL97 used are D14980 and U07355 of the Towne strain and X17403 of the AD169 strain.

CASE REPORT

A 54-year-old man with myelodysplastic syndrome of refractory anemia with excess blasts (RAEB) with cytogenetic abnormalities including der(1;7)(q10;p10) participated in a clinical study of 2- or 3-locus- mismatched hematopoietic stem cell transplantation using in vivo alemtuzumab, because he did not have an available HLA-A/B/DR-matched related donor, 1-locus-mismatched related donor, or an HLA-matched unrelated donor. He had repeated episodes of infectious disease including pulmonary invasive aspergillosis due to sustained neutropenia. He was CMV-seropositive, but the donor was CMV-seronegative. The conditioning regimen consisted of alemtuzumab (0.2 mg/kg/day × 6 days, from day 8 to day 3), fludarabine (30 mg/m^2 × 6 days, from day 8 to day 3), busulfan (4 mg/kg/day × 2 days, from day 5 to day 4), and total body irradiation (2 Gy twice daily on day 1). Peripheral blood mononuclear cells were collected from his 3-locus-mismatched daughter, cryopreserved without ex vivo manipulation, and infused on day 0. The number of infused CD34 and CD3 positive cells was 5.24×10^6 cells/kg and 2.72×10^8 cells/kg of the recipient body weight, respectively. Post-transplantation prophylaxis against graft-versus-host disease was performed with continuous infusion of cyclosporine A (3 mg/kg) and short-term methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11). Neutrophil engraftment, defined as the first of 3 consecutive days with an absolute neutrophil count of at least 0.5×10^9/L, was documented on day 12. Though febrile neutropenia was observed for 9 days, no bacterial and fungal infection was documented during neutropenia. Acute graft-versus-host disease was not clinically observed.

CMV reactivation was first detected on day 19 after transplantation by the CMV antigenemia assay that was performed weekly after engraftment using C10/C11 antibody. Preemptive antiviral treatment with intravenous ganciclovir (5 mg/kg twice daily) was initiated [Kanda et al., 2001b]. The level of antigenemia was decreased gradually and the dose of ganciclovir was decreased to 5 mg/kg/day. However, antigenemia increased again, which was refractory to the re-increase in the dose of ganciclovir to 5 mg/kg twice daily. Antiviral agents were changed from ganciclovir to foscarnet at 50 mg/kg twice daily adjusted to his renal function at day 72 after transplantation, that was temporarily effective but antigenemia increased again